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(71) Applicant: CHILDREN'S BIOMEDICAL RESEARCH INSTITUTE [US/US]; 345 North Smith Avenue, St. Paul, MN 55102 (US).

(72) Inventors: GEHRZ, Richard, C. ; 1047 Marie, Mendota Heights, MN 55118 (US). KARI, Bruce, E. ; 3218-38th Avenue South, Minneapolis, MN 55406 (US). LUSSENHOP, Nancy, O. ; 1757 Scheffer Avenue, St. Paul, MN 55116 (US).

(74) Agent: HAMRE, Curtis, B.; Merchant, Gould, Smith, Edell, Welter & Schmidt, 3100 Norwest Center, 90 South Seventh Street, Minneapolis, MN 55402 (US).

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(54) Title: HUMAN CYTOMEGALOVIRUS-SPECIFIC MONOCLONAL ANTIBODY COCKTAIL

(57) Abstract

Disclosed is a pharmaceutical composition and a method for its use in the diagnosis and medical treatment of human cytomegalovirus infection. The immunogenic cocktail composition combines a mixture of at least two HCMV-specific immunoglobulins with a suitable carrier.

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HUMAN CYTOMEGALOVIRUS-SPECIFIC MONOCLONAL ANTIBODY COCKTAIL

Field of Invention

The present invention is directed to a number of pharmaceutical compositions suitable for diagnosis or therapeutic treatment of human cytomegalovirus (HCMV) infections, to methods of medical diagnosis and treatment of HCMV infections, and to a method of making compositions utilized in connection with HCMV infection.

Background of the Invention

Human cytomegalovirus (HCMV) is a ubiquitous virus and measures to limit exposure to HCMV have had little impact on the incidence or severity of congenital or opportunistic HCMV infections. A large number of asymptomatic HCMV-infected children and adults are known to episodically excrete HCMV in their body secretions and there is no known practical way to limit contact between them and individuals who are highly susceptible to HCMV infection, such as seronegative pregnant women and immunosuppressed patients.

HCMV is the most common cause of congenital and opportunistic infections associated with acquired immune deficiency syndrome (AIDS) and with patients undergoing organ and bone marrow transplants. Rejection of graft material is also frequently associated with HCMV infection. HCMV infection of a developing fetus may retard intrauterine growth and cause congenital complications such as chorioretinitis, microcephaly, intracranial calcifications, psychomotor retardation and multiple neurological defects. HCMV infections in human immunodeficiency virus (HIV)-seropositive individuals result in such complications as chorioretinitis, hepatitis, hemorrhagic gastroenteritis, and progressive interstitial pneumonia. HCMV has been implicated in the pathogenesis of peripheral neuritis and progressive encephalomyelopathy associated with HIV infections of the central nervous system and Kaposi's sarcoma. HCMV has also been implicated as a co-factor in the

development of overt AIDS by causing immunosuppression of high-risk HIV-seronegative individuals which predisposes them either to primary HIV infection or to reactivation of latent HIV, or by providing complimentary genetic information that increases the replication of HIV in cells which are co-infected with HCMV.

Exposure of HCMV-seronegative transplant recipients to HCMV-infected donor organs or blood products may result in symptomatic HCMV infections which lead to interstitial pneumonia, leukopenia, chorioretinitis and other life-threatening complications. The use of HCMV-seronegative blood products, organs and bone marrow significantly reduces the risk of HCMV infection. However, HCMV-seropositive donors cannot be excluded in all cases because of the limited supply of appropriately matched donor organs and bone marrow and the urgency of most heart and liver transplants. HCMV infection in seropositive transplant recipients is the consequence of their immunosuppressed condition in conjunction with the reactivation of latent HCMV virions.

Prophylactic administration of anti-viral chemotherapy or HCMV hyperimmune globulin or plasma prior to and following organ and bone marrow transplants has met with limited success. The reason is that HCMV hyperimmune globulin is active against extracellular HCMV present in the early stage of infection but may not be active against late stage intracellular virion replication and/or cell-to-cell spread. As a result, HCMV-hyperimmune globulin provides some protection for an HCMV-seronegative patient receiving bone marrow or an organ from an HCMV-seropositive donor, but has little effect in inhibiting the reactivation of latent intracellular virions in HCMV-seropositive individuals.

Existing treatments of patients with established HCMV infections have also had limited

success. HCMV has a slow replication cycle compared to that of other herpes viruses and is highly cell-associated which makes the virus relatively inaccessible to humoral antibodies. Moreover, HCMV uses certain host cell enzymes for viral replication rather than virus-specific enzymes which can be interdicted by certain anti-viral drugs. HCMV hyperimmune globulin primarily contains antibodies directed at substances other than HCMV as well as antibodies that may protect the virus at the expense of the host. Therefore, with that treatment only a small proportion of the total antibody protein is effective against HCMV. A further disadvantage of human immune globulin preparations is its potential for transmitting infectious agents such as hepatitis or the human immunodeficiency virus (HIV). Likewise, the administration of a single type of monoclonal antibody (mcAb) is not necessarily effective against all naturally occurring strains of HCMV or in cases where a patient is infected with multiple strains of HCMV. It can also be expected that a virus like HCMV will mutate into resistent strains in response to the prolonged administration of a single mcAb. Existing murine antibody therapies also present the risk that the patient will develop human anti-mouse antibodies (HAMA) in response to the therapeutic mcAb.

In order to identify effective treatments against HCMV, it is important to understand the structure of the HCMV virus. The most abundant HCMV envelope glycoproteins and glycoprotein complexes are summarized in Table 1 shown below.

TABLE 1

Summary of Human Cytomegalovirus Envelope Glycoproteins and Complexes

Complexe(es)	MW(daltons)	Glycoprotein(s)	MW(daltons)	Gene(s)	References
	130,000	gp130	130,000	gB	1,3,4,7
gCI	180,000	gp93	93,000	U_L	
	>450,000	gp55	55,000	(.344-.360)	
gCII	93,000	Group 1 gp47-52	47,000 - 52,000	HXLF	1,2,3,5,6
	>200,000	Group 2 gp38-47	38,000 - 47,000	(1-5)	
		gp90	90,000	U_S	
		gp200	>200,000	(.857-879)	

References

1. Kari, B., N. Lussenhop, R. Goertz, M. Wabuke-Bunoti, R. Radeke, and R. Gehrz. Characterization of monoclonal antibodies reactive to several biochemically distinct human cytomegalovirus glycoprotein complexes. J. Virol. 60:345-352 (1986).

2. Gretch, D.R., B. Kari, R. Gehrz, and M. Stinski. A multigene family encodes the human cytomegalovirus glycoprotein complex gCII (gp47-52 complex). J. Virol. 62:1956-1962 (1988).

3. Gretch, D.R., B. Kari, L. Rasmussen, R. Gehrz, and M. Stinski. Identification and characterization of three distinct families of glycoprotein complexes in the envelopes of human cytomegalovirus. J. Virol. 62:875-881 (1988).

4. Gretch, D.R., R. Gehrz, and M. Stinski. Characterization of a human cytomegalovirus glycoprotein complex (gCI). J. Gen. Virol. 69:1205-1215 (1988).

5. Kari, B. and R. Gehrz. Isolation and characterization of a human cytomegalovirus glycoprotein containing a high content of O-linked oligosaccharides. Arch. Virol. 98:171-188 (1988).

Table 1 Cont'd

6. Kari, B., R. Goertz, N. Lussenhop, and R. Gehrz. Characterization of a family of human cytomegalovirus glycoprotein complexes designated gC-II with murine monoclonal antibodies and human sera. Submitted, J. Gen. Virol.

7. Kari, B., R. Goertz, N. Lussenhop, and R. Gehrz. Characterization of neutralizing murine monoclonal antibodies recognizing a 93,000 molecular weight glycoprotein in human cytomegalovirus glycoprotein complex I (gC-I). Submitted, J. Virol.

The gCI family of glycoprotein complexes (gC) contains glycoproteins (gp) with molecular weights of 130,000 (gp130), 93,000 (gp93), and 55,000 (gp55). These glycoproteins have been designated as gB glycoproteins based on extensive amino acid sequence homology with the gB glycoproteins of herpes simplex virus (HSV) and Epstein-Barr virus (EBV), and gCII of varicella zoster virus (VZV). These glycoproteins derive from a single coding region in the unique long (U_L) region of the HCMV genome, which may represent a primordial gene conserved among herpes viruses involved in biological functions critical to the evolution and survival of these viruses. Two mature glycoproteins, gp55 and gp93, may represent the carboxy-terminal and amino-terminal components of a fully glycosylated precursor, respectively, resulting from a single proteolytic cleavage event. A third glycoprotein, gp130, may result from a minor carbohydrate modification of the precursor without proteolysis. These glycoproteins exist in virion envelopes primarily in the form of disulfide-linked complexes. The glycoproteins gp130 and gp55 were originally identified as gA by Pereira et al., and claimed in U.S. Patent No. 4,689,225, issued August 8, 1987, the disclosure of which is incorporated by reference herein. gp93 has been identified as a glycoprotein unique from gp55 and gp130, and unique murine mcAbs reactive with gp93 have been described in pending U.S. Patent Application Serial No. 07/407,276, filed on September 14, 1989, the disclosure of which is incorporated by reference herein. The unreduced gCI complex with a molecular weight of 130,000 contains gp130 and gp55, the gCI complex with a molecular weight of 180,000 contains gp130 and gp93, and the high molecular weight gCI complexes contain all three glycoproteins.

A second family of glycoprotein complexes designated as gCII is comprised of an abundant

glycoprotein of 47,000 - 52,000 (gp47-52) and a group of glycoproteins with molecular weights of 38,000-47,000, 90,000 and greater than 200,000. gp47-52 is the product of a bicistronic mRNA transcript encoded by the HXLF 1 and 2 genes of a homologous gene family in the Hind III F fragment of the unique short (U_s) region of the HCMV genome. The unreduced gCII complex with a molecular weight of 93,000 is comprised of multimer (a covalently bonded combination of several polymeric molecules) of a single glycoprotein, gp47-52, recognized by the family of mcAbs designated as Group 1 gCII mcAbs as claimed in U.S. Patent Application Serial No. 07/390,300, filed on August 7, 1989, the disclosure of which is incorporated by reference herein. A higher molecular weight unreduced gCII complex with a molecular weight of greater than 200,000 contains antigenically distinct glycoproteins gp38-47, gp90 and gp200, recognized by Group 2 gCII mcAbs claimed in U.S. Patent Application Serial No. 07/390,300, filed on August 7, 1989, the disclosure of which is incorporated by reference herein, as well as gp47-52 recognized by Group 1 mcAbs. The complex molecular organization of the HXLF gene family and the polymorphism of its related gCII glycoproteins suggest that HCMV has evolved these glycoproteins for some essential biological function.

These glycoproteins and glycoprotein complexes are important in maintaining the structural integrity of the virion, and also play an essential role in biological processes essential to viral infectivity (i.e., adsorption, penetration), virion assembly, and cell-to-cell spread. Since currently available means of prevention and treatment of HCMV-related infections are of limited benefit, a goal of the present invention is the development of a pharmaceutical composition that can significantly reduce the incidence and severity of HCMV infections. A particular objective of the invention is the production of a pharmaceutical composition that is

active against HCMV in both the extracellular phase or early stage of HCMV infection and the intracellular phase or late stage of HCMV infection. Such a composition would be useful for treating individuals with established HCMV infections as well as for prophylaxis for those who are highly susceptible to HCMV infection. Yet another goal is the development of a composition which is active against virtually all strains of HCMV and will forestall the development of resistent strains of HCMV. A further goal is to develop a pharmaceutical composition active against HCMV which will reduce the risk of HAMA response in a patient.

Summary of the Invention

These and other goals are met by the present invention which is directed to an immunogenic cocktail composition and a method for its use in medical treatment of HCMV infection. More specifically, the cocktail composition, which is suitable for both diagnostic and therapeutic use, constitutes a mixture of at least two HCMV-specific immunoglobulins.

All immunoglobulins selected for the mixture bind with HCMV envelope glycoproteins and/or their complexes. Generally, the cocktail contains at least two anti-viral immunoglobulins each of which binds with a different segment of HCMV envelope glycoproteins or complexes. Preferably, these segments incorporate different, unique, non-competing epitopes on the same or different HCMV envelope glycoproteins. This function produces cocktail reactivity with multiple strains of HCMV. Preferably within the mixture, the immunoglobulins, at least in part, exhibit synergistic anti-viral neutralizing activity against HCMV and are active against different phases of HCMV infection.

It is especially preferred that at least one of the immunoglobulins of the mixture will enhance the neutralizing activity of another member of the mixture

and those or other members will exhibit complement-dependent and complement-independent anti-viral activity, and will be active against intracellular and extracellular HCMV glycoproteins.

A preferred embodiment of the mixture combines three immunoglobulins, two being the synergistic pair of which one provides complement-dependent anti-viral activity, and the third providing complement-independent anti-viral activity.

Another preferred embodiment of the mixture combines four immunoglobulins, two providing synergistic anti-viral activity against HCMV, a third exhibiting complement-dependent anti-viral activity, and a fourth providing complement-independent anti-viral activity.

Immunoglobulins which are suitable for the composition of the invention include HCMV-specific antibodies such as murine monoclonal antibodies (mcAbs), immunogenic fragments thereof such as Fab, $F(ab')_2$ and their mixture with Fc, human monoclonal antibodies, chimeric antibodies carrying human constant regions, and altered human antibodies carrying hypervariable segments derived from HCMV-specific antibodies from a differing mammalian source. Murine mcAbs are among those preferred.

A highly preferred embodiment of the composition of the invention is the mixture of four murine mcAbs designated as MM-HCMV-9B7, MM-HCMV-41C2, MM-HCMV-F15-3B10, and MM-HCMV-15F9 (In Vitro International deposit nos. IVI-10117, IVI-10119, IVI-10207, and IVI-10182, respectively). (MM refers to an internal code designation of the manufacturer, Medimorphics).

When the cocktail composition is to be employed as a pharmaceutical composition for therapeutic and prophylactic treatment of HCMV infection in humans, it will be composed of a therapeutically effective amount of the foregoing immunoglobulin mixture with a

pharmaceutically-acceptable carrier. The carrier is preferably a liquid.

When the cocktail composition is to be employed for diagnostic purposes such as in vitro analysis, it will be composed of appropriate diagnostic amounts of the foregoing immunoglobulin mixture, optional suitable diluents whether or not pharmaceutically acceptable and optionally, detection markers or tags for measurement of the amount of HCMV detected.

The invention is further directed to a method of therapeutic and prophylactic medical treatment for HCMV infection which involves administering a therapeutically effective amount of pharmaceutical composition to a patient.

The invention is also directed to a method of making the immunogenic cocktail composition which involves selecting compatible immunoglobulins which manifest certain predetermined activity against HCMV, and combining the immunoglobulins with a suitable carrier. In the case of a pharmaceutical composition, a pharmaceutically-acceptable carrier is combined with the mixture. Preferably, the carrier is a liquid.

Detailed Description of the Invention

The cocktail composition of the present invention combines several HCMV-specific immunoglobulins. This cocktail provides a preparation useful for the treatment of early and/or late HCMV infections in humans caused by virtually all strains of HCMV.

The immunoglobulins useful in the mixture may be derived from various sources, the preferred type being monoclonal antibodies. Tables 2-8 outline examples of mcAbs for the mixture. Monoclonal antibodies for a particular mixture may be derived from hybridomas or other cell lines (i.e., EBV-transformed lymphoblastoid cell lines, transfected mammalian cell

lines expressing immunoglobulin genes) that generate human, chimeric, bi-specific rearranged and murine antibodies. The unique specificity and functional activity of the murine HCMV-specific mcAbs in the cocktail have been precisely characterized and those structural features of the murine mcAbs that provide the specificity and activity can be engineered into chimeric human-mouse mcAbs exhibiting similar therapeutic advantages. Alternatively, hybridomas producing human antibodies of similar specificity can be obtained by immunizing and/or screening with purified HCMV peptides recognized by the murine mcAbs in the cocktail. Methods to prepare these antibodies have been previously described in U.S. Patent Nos. 4,816,397 and 4,816,567. In addition, F(ab) or $F(ab')_2$ fragments may be utilized alone or in combination with Fc fragments. For methods to prepare these immunogenic fragments, see, for example, Darnell et al., Molecular Cell Biology, Scientific American, Inc., New York, NY (1986) at pages 77-78.

It is preferred that the immunoglobulin members of the cocktail react with as many naturally occurring strains of HCMV as possible while exhibiting no cross-reactivity with other viruses or normal cellular proteins. It is further preferred that the cocktail contain at least two different anti-viral immunoglobulins that are reactive with unique, non-competing epitopes on the same or different HCMV envelope glycoproteins. In addition to the foregoing benefits, this structure and activity relationship will immunoreact with existing strains of HCMV and prevent the emergence of future strains of HCMV which would be otherwise resistent to the anti-viral activity of an immunoglobulin mixture.

Several factors are taken into consideration in selecting a particular immunoglobulin for the mixture. The cocktail comprises an immunoglobulin mixture that

exhibits specificity for multiple epitopes, augmenting activity, and synergistic anti-viral activity directed against mechanisms associated with both extracellular viral infection and established intracellular replicating viral infection. A single immunoglobulin may function in more than one capacity. A particular immunoglobulin is thus selected on the basis of its reactivity with specific epitopal sites on an HCMV glycoprotein complex or component glycoprotein, its synergistic activity in combination with other immunoglobulins, its complement-dependent or complement-independent anti-viral activity against HCMV, and its activity against early stage extracellular HCMV or late stage intracellular HCMV.

Immunoglobulins used in the cocktail are reactive with HCMV envelope glycoproteins. Since those glycoproteins are expressed on the surface of HCMV virions as well as on the membranes and in the cytoplasm of cells which have become infected with the virus, the preparation provides neutralizing activity against both extracellular and intracellular HCMV virions.

The selection of a particular immunoglobulin depends on the type of anti-viral activity sought. In particular, the cocktail contains at least two HCMV-specific immunoglobulins that exhibit anti-viral activity by different neutralizing actions wherein the activity is effective against different stages of HCMV infection. It is preferred that the cocktail combine at least two monoclonal antibodies with different anti-viral activity selected from the following types of neutralizing actions:

Neutralizing Action	Characteristics of mcAbs
(i) Lysis of extracellular infectious virus	Complement-fixing mcAbs reactive with neutralizing epitopes on HCMV envelope glycoproteins and complexes

(ii) Inhibition of virus adsorption or penetration into host cells	Blocking mcAbs which react with HCMV envelope glycoproteins and complexes essential for adsorption and/or fusion with cell membranes
(iii) Inhibition of virion assembly, cell-to-cell spread	Inhibitory mcAbs reactive with essential structural HCMV envelope glycoproteins
(iv) Lysis of HCMV-infected host cells	
(a) antibody-mediated cytolysis	Complement-dependent or -independent cytolytic mcAbs reactive with HCMV glycoproteins and complexes on the surface of infected cells which directly lyse the virus-infected cell
(b) cell-mediated cytoxicity	Complement-dependent or -independent mcAbs which mediate lymphocyte killing of infected cells (i.e., antibody-dependent cellular cytoxicity (ADCC))

More particularly, immunoglobulins are combined which react with different glycoprotein complexes (gC) and with different glycoproteins (gp) within those complexes. The most abundant HCMV envelope glycoproteins and glycoprotein complexes are summarized in Table 1.

Various neutralizing immunoglobulins specific to the gCI glycoprotein complexes inactivate extracellular HCMV, block viral adsorption to target organs, and/or prevent penetration of HCMV into tissues. The gCI-specific immunoglobulins include those which are reactive with glycoproteins with molecular weights of 130,000 (gp130), 93,000 (gp93) and 55,000 (gp55). Among the gCI-specific mcAbs preferred are those outlined in Table 2 and Table 3, as shown below.

TABLE 2

Characterization of gc-I Monoclonal Antibodies Reactive with gp130/55

	McAb	Neutralization with Complement	IF[a]	IP of gc-I Complexes[b]	WB of gc-I Glycoproteins[c]
Domain I	MM-HCMV-41C2	-	+	+	gp130/55
	MM-HCMV-26B11	-	+	+	-
	MM-HCMV-39E11	+	+	+	gp130/55
Domain II	MM-HCMV-9B7	+	+	+	gp130/55
	MM-HCMV-18F9	+	+	+	gp130/55
	MM-HCMV-34G7	-	+	+	gp130/55
Domain III	MM-HCMV-11B4	-	+	+	-
	MM-HCMV-23B11	-	+	+	-

[a] IF = indirect immunofluorescence of HCMV-infected fibroblasts

[b] IP = immunoprecipitation of unreduced gc-I glycoprotein complexes

[c] WB = Western blot analysis of reduced gc-I glycoproteins; non-reactive mcAbs recognize conformational determinants

TABLE 3

Characterization of gc-I Monoclonal Antibodies Reactive with gp130/93

McAb	Neutralization without Complement	IF[a]	IP of gc-I Complexes[b]	WB of gc-I Glycoproteins[c]
MM-HCMV-3C2	+	+	+	gp130/93
MM-HCMV-9F9	+	+	+	gp130/93
MM-HCMV-F15-3B10	+	+	+	gp130/93

[a] IF = indirect immunofluorescence of HCMV-infected fibroblasts

[b] IP = immunoprecipitation of unreduced gc-I glycoprotein complexes

[c] WB = Western blot analysis of reduced gc-I glycoproteins

According to one embodiment of the invention, the gCI-specific immunoglobulins include MM-HCMV-9B7, a gp55-specific neutralizing IgG_{2b} mcAb (deposit no. IVI-10117), MM-HCMV-41C2, a gp55-specific non-neutralizing IgG_1 mcAb (deposit no. IVI-10119), and MM-HCMV-F15-3B10, a gp93-specific neutralizing IgG_1 mcAb (deposit no. IVI-10207). (MM refers to an internal code designation of the manufacturer, Medimorphics).

The further inclusion of an immunoglobulin specific to the gCII complex may inhibit HCMV adsorption and penetration of tissues, intracellular virion assembly, and/or cell-to-cell spread. Immunoglobulins specific to the gCII complex include Group 1 gCII immunoglobulins which are reactive with gp47-52 and recognize membrane-associated glycoproteins, and Group 2 gCII immunoglobulins which are recognize gp38-47, gp90 and gp200 of gCII and exhibit activity against intracellular proteins. Among the gCII-specific mcAbs preferred are those listed in Table 4, as shown below.

TABLE 4

Characterization of gc-II Monoclonal Antibodies Reactive with Group 1 and 2 Glycoproteins

	McAb	Neutralization without Complement	IF[a]	IP of gc-II Complexes[b]	WB of gc-II Glycoproteins
Group 1	MM-HCMV-9E10	+	+	+	gp47-52
	MM-HCMV-8B4	-	+	+	gp47-52
	MM-HCMV-8B2	-	+	+	gp47-52
	MM-HCMV-26E11	-	+	+	gp47-52
Group 2	MM-HCMV-12G9	-	+	+	gp38-47/90/200
	MM-HCMV-14F9	-	+	+	gp38-47/90/200
	MM-HCMV-15F9	-	+	+	gp38-47/90/200
	MM-HCMV-15C7	-	+	+	gp38-47/90/200
	MM-HCMV-23B10	-	+	+	gp38-47/90/200
	MM-HCMV-25B2	-	+	+	gp38-47/90/200
	MM-HCMV-25C8	-	+	+	gp38-47/90/200
	MM-HCMV-27B4	-	+	+	gp38-47/90/200
	MM-HCMV-40B4	-	+	+	gp38-47/90/200

[a] IF = indirect immunofluorescence of HCMV-infected fibroblasts

[b] IP = immunoprecipitation of unreduced gc-II glycoprotein complexes

[c] WB = Western blot analysis of reduced gc-II glycoproteins

According to one embodiment of the invention, Group 1 gCII-specific immunoglobulins include MM-HCMV-9E10, a gp47-52-specific neutralizing mcAb (deposit no. IVI-10118), and MM-HCMV-8B4, MM-HCMV-8B2, and MM-HCMV-26E11, three gp47-52-specific non-neutralizing mcAbs. Group 2 gCII-specific immunoglobulins include, among others, MM-HCMV-15F9, a non-neutralizing mcAb specific to gp38-47, gp90 and gp200 (deposit no. IVI-10182).

According to the invention, it is preferred that immunoglobulins specific to gp55 and gp93 of the gCI glycoprotein complexes are combined with immunoglobulins which react with gp38-47, gp90 and gp200 of the gCII complexes containing Group 2 glycoproteins. The antigen specificity and the anti-viral activity of such preferred mcAbs selected for the mixture of an HCMV-specific mcAb cocktail, as summarized in Table 5 below.

TABLE 5

Specificity of Monoclonal Antibody Members of an HCMV-Specific Monoclonal Antibody Cocktail for Unique HCMV Envelope Glycoproteins and Complexes

Monoclonal Antibody	Antigen Specificity	Anti-Viral Activity	References
MM-HCMV-9B7	gp130/gp55 of gCI	Complement-dependent neutralization	1,2,4,5,7,10
MM-HCMV-41C2	gp130/gp55 of gCI	Augmentation of 9B7-neutralization	1,2,4,5,6,10
MM-HCMV-F15-3B10	gp130/gp93 of gCI	Complement-independent neutralization	1,4,5,7,9
MM-HCMV-15F9	gp38-47/gp90/gp200 (Group 2) of gCII	Inhibition of viral infectivity/replication	1,3,6,7,8

References

1. Kari, B., N. Lussenhop, R. Goertz, M. Wabuke-Bunoti, R. Radeke, and R. Gehrz. Characterization of monoclonal antibodies reactive to several biochemically distinct human cytomegalovirus glycoprotein complexes. J. Virol. 60:345-352 (1986).

2. Lussenhop, N.O., R. Goertz, M. Wabuke-Bunoti, R. Gehrz, and B. Kari. Epitope analysis of human cytomegalovirus glycoprotein complexes using murine monoclonal antibodies. Virology 164:362-372 (1988).

3. Gretch, D.R., B. Kari, R. Gehrz, and M. Stinski. A multigene family encodes the human cytomegalovirus glycoprotein complex gCII (gp47-52 complex). J. Virol. 62:1956-1962 (1988).

Table 5 Cont'd

4. Gretch, D.R., B. Kari, L. Rasmussen, R. Gehrz, and M. Stinski. Identification and characterization of three distinct families of glycoprotein complexes in the envelopes of human cytomegalovirus. J. Virol. 62:875-881 (1988).

5. Gretch, D.R., R. Gehrz, and M. Stinski. Characterization of a human cytomegalovirus glycoprotein complex (gCI). J. Gen. Virol. 69:1205-1215 (1988).

6. Kari, B., and R. Gehrz. Isolation and characterization of a human cytomegalovirus glycoprotein containing a high content of O-linked oligosaccharides. Arch. Virol. 98:171-188 (1988).

7. Liu, Y-N.C., B. Kari, and R. Gehrz. Human immune responses to major human cytomegalovirus complexes. J. Virol. 62:1066-1070 (1988).

8. Kari, B., R. Goertz, N. Lussenhop, and R. Gehrz. Characterization of a family of human cytomegalovirus glycoprotein complexes designated gC-II with murine monoclonal antibodies and human sera. Submitted, J. Gen. Virol.

9. Kari, B., R. Goertz, N. Lussenhop, and R. Gehrz. Characterization of neutralizing murine monoclonal antibodies recognizing a 93,000 molecular weight glycoprotein in human cytomegalovirus glycoprotein complex I (gC-I). Submitted, J. Virol.

10. Gehrz, R.C., B. Kari, Y-N.C., Liu, and M. Stinski. Immune recognition of HCMV envelope glycoproteins. Presented, 2nd International Cytomegalovirus Workshop (1989).

At least one immunoglobulin specific to the gCI glycoprotein complex of HCMV may be selected for its complement-dependent neutralizing activity. The complement-dependent neutralization of HCMV may involve extracellular lysis of virions. Preferably this neutralizing activity is directed against a complement-dependent epitope of gp55 of the gCI glycoprotein complexes. Among the embodiments of those mcAbs preferred for this activity are MM-HCMV-39E11, MM-HCMV-18F9, and MM-HCMV-9B7, as characterized in Table 2. According to an embodiment of the invention, MM-HCMV-9B7, a murine IgG_{2b} mcAb (deposit no. IVI-10117), can be selected for the mixture for its complement-dependent neutralizing activity with an epitope on gp55.

At least one immunoglobulin which exhibits complement-independent neutralizing activity toward the gCI glycoprotein complexes of HCMV may also be selected for the mixture. This activity may interfere with HCMV infectivity by blocking adsorption to viral receptors expressed on target organs and by preventing the penetration of HCMV into susceptible cells. Among those mcAbs preferred for this activity are MM-HCMV-3C2, MM-HCMV-9F9, and MM-HCMV-F15-3B10, all of which are specific to complement-independent epitopes on gp93 of the gCI glycoprotein complexes, as characterized in Table 3. For this particular member of the mixture, an embodiment of the invention can include MM-HCMV-F15-3B10, a murine IgG_1 mcAb.

At least one immunoglobulin may be incorporated into the mixture based on its reactivity against Group 2 glycoproteins in gcII glycoprotein complexes of HCMV. Reactivity against gCII glycoprotein complexes may interfere with late stage intracellular HCMV by preventing virion assembly and/or cell-to-cell spread of the virus. Table 4 outlines Group 2 gCII mcAbs which are among those preferred for this activity. An embodiment of this kind of mcAb is MM-HCMV-15F9, a

murine IgG_{2a} mcAb (deposit no. IVI-10182) which is reactive with gp38-47, gp90 and gp200 of the gCII glycoprotein complexes containing Group 2 glycoproteins.

At least one immunoglobulin is selected to inactivate extracellular HCMV associated with the early stage of infection and at least one immunoglobulin is selected to inhibit intracellular HCMV once infection of tissues has occurred. The combination of immunoglobulins which neutralize or inhibit HCMV in different stages of infectivity increases the effectiveness of a preparation directed at inhibiting HCMV infection over a wide range of situations.

According to the invention, early stage antiviral activity against HCMV is provided by gCI-specific immunoglobulins with complement-dependent and complement-independent neutralizing activity while late stage inhibitory activity against intracellular HCMV is provided by Group 2 gCII-specific immunoglobulins. In a preferred embodiment of the invention, MM-HCMV-9B7, a murine IgG_{2b} mcAb specific to gp55 of the gCI glycoprotein complex of HCMV, can provide complement-dependent neutralization which involves extracellular lysis of HCMV virions. MM-HCMV-F15-3B10, a complement-independent murine IgG_1 mcAb specific to gp93 of the gCI complex, may also interfere with early stage exogenous HCMV by inhibiting viral adsorption and penetration into susceptible cells. The Group 2 gCII-specific murine IgG_{2a} mcAb MM-HCMV-15F9 may inhibit intracellular virion assembly and/or cell-to-cell spread of HCMV.

At least two immunoglobulins are selected because of their synergistic enhancement of neutralizing activity against HCMV that occurs when the immunoglobulins are combined. It is preferred that the immunoglobulins are reactive with unique, augmenting epitopes on the same or different HCMV envelope glycoproteins such that the anti-viral activity of the immunoglobulins in combination is substantially greater

than the sum of the activities of each immunoglobulin acting alone. The augmenting activity may result from the molecular interactions between immunoglobulins reactive with epitopes in close spatial proximity on the conformational structure of a single glycoprotein or from interactions between immunoglobulins directed against different glycoproteins. It is preferred that at least one immunoglobulin react with a non-neutralizing epitope to cause conformational changes in the three-dimensional structure of the glycoprotein which in turn increases the accessibility of the second immunoglobulin toward a neutralizing epitope. Preferably the neutralizing activity is directed at a complement-dependent epitope of the gCI glycoprotein complexes. The result of the combined activity of the immunoglobulins is an increase in the complement-dependent activity of the neutralizing immunoglobulin.

This synergistic activity may be provided by combining mcAbs specific to Domain I of the gCI glycoprotein complexes with mcAbs specific to Domain II of the gCI glycoprotein complexes. Among those Domain I and II mcAbs that are preferred are those outlined in Table 2. According to the invention, an embodiment providing this activity may be two mcAbs specific to gp55 on the gCI glycoprotein complexes of HCMV. These mcAbs are selected for their synergistic immunoreactivity and neutralizing activity when combined. In particular, MM-HCMV-41C2, a murine IgG_1 mcAb (deposit no. IVI-10119), can be selected for its non-neutralizing activity with an epitope located in close spatial proximity on the conformational structure to the epitope recognized by MM-HCMV-9B7 in order to augment the complement-dependent neutralizing activity of MM-HCMV-9B7 and enhance extracellular lysis of HCMV virions.

Representative sources of monoclonal antibodies reactive with HCMV envelope glycoproteins are provided in Tables 6 and 7 shown below.

TABLE 6

Representative Sources of Monoclonal Antibodies Reactive with HCMV Envelope Glycoproteins Disclosed in the Literature

Laboratory Investigator/Location	McAbs	Specificity	Source	Functional Activity	Reference
Kari et al. CBRI, St. Paul (Medimorphics)	8	gp130/gp55 of gcI	mouse	°complement-dependent neutralization °augmentation of anti-viral activity	1,2
	2	gp130/gp93 of gcI	mouse	complement-independent neutralization	3
	4	gp47-63 (group 1) of gcII	mouse	°complement-independent neutralization °inhibition of virus infectivity, virion assembly, cell-to-cell spread	1,3
	9	gp38-47/gp90/ gp200 (group2) of gcII	mouse	°inhibition of virus infectivity, virion assembly, cell-to-cell spread	3
Pereira et al. Viral & Rickettsial Disease Lab. Cal.	16	gp130/gp55 of gcI	mouse	complement-dependent & independent neutralization	4,5
Britt et al. U of Alabama (Birmingham)	5	gp130/gp55 of gcI	mouse	complement-dependent neutralization	6,7

Table 6 cont'd

REFERENCES:

1. J. Virol. 60:345, 1986.
2. Virology 164:362, 1988.
3. Submitted for publication.
4. Infect. Immunity 36:924, 1982.
5. Virology 139:73, 1984.
6. Virology 135:369, 1984.
7. J. Virol. 58:185, 1986.

TABLE 7

Laboratory Investigator/Location	McAbs	Specificity	Source	Functional Activity	Reference
Rasmussen et al. Stanford (Calif.)	1	gp86 of gcIII	mouse	complement-independent neutralization	1,2
	2	gp130/gp55 of gcI	mouse	complement-dependent neutralization	2,3
Nowak et al. (Institute for Klinische Virologie, FRG)	1	gp130/gp55 of gcI	mouse	--	4
Law et al. Center for Applied Microbiol, Salisbury, UK	1	gp130/gp55 of gcI	mouse	--	5
Kim (New York Institute for Basic Research, Staten Island, NY)	5	?gp66	mouse	?	6
Emanuel et al. (Sloan-Kettering, NY)	1	?specificity	human,by EBV-B cell x human LCL	?	7
Redmond et al. U of Alberta, Canada	6	?p 66,95 (?gcI)	human by EBV transfor-mation	complement-independent neutralization	8
Masuho et al. Teijin, Japan	6	gp130/gp55 of gcI	human-mouse hetero-hybridoma	complement-independent and dependent neutralization	9

Table 7 Cont'd

REFERENCES

1. Proc. Natl. Acad. Sci. (USA) 81:876, 1984.
2. Virology 163:308, 1988.
3. J. Virol. 55:274, 1985.
4. Virology 132:325, 1984.
5. J. Med. Virol. 17:255, 1985.
6. J. Clin. Microbiol. 18:331, 1983.
7. J. Immunol. 133:2202, 1984.
8. J. Virol. Meth. 14:9, 1986.
9. J. Gen. Virol. 68:1457, 1987.

Hybridomas producing HCMV-specific mcAbs that are deposited at In Vitro International, Inc. are listed in Table 8 shown below.

TABLE 8

B Cell Hybridomas Producing HCMV-Specific Monoclonal Antibodies Deposited at In Vitro International, Inc.

CBRI Designation	Medimorphics Designation	IVI Deposit No.	Specificity
9B7	MM-HCMV-9B7	IVI-10117	gp130/55 of gC-I complexes
41C2	MM-HCMV-41C2	IVI-10119	gp130/55 of gC-I complexes
34G7	MM-HCMV-34G7	IVI-10142	gp130/55 of gC-I complexes
F15-3B10	MM-HCMV-F15-3B10	IVI-10207	gp130/93 of gC-I complexes
9E10	MM-HCMV-9E10	IVI-10118	gp47-52 (Group I) of gC-II complexes
15F9	MM-HCMV-15F9	IVI-10182	gp38-47/90/200 of gC-II complexes
11B4	MM-HCMV-11B4	IVI-10181	--

Different formulations of the cocktail can be achieved by mixing the mcAbs in different proportions and combining the mixture with the carrier. It is preferred that a range of ratios and immunoglobulin concentrations of various combinations of immunoglobulins be evaluated according to microneutralization assay and the proportion of a mcAb member within the mixture be selected from the corresponding range of augmenting anti-viral activity. For a mixture of two mcAbs, it is preferred that ranges of 100:1 to 1:100 be evaluated. According to the invention, a 10-100 fold increase in the neutralizing activity has been observed in 9B7:41C2 over a range of 1:10 to 1:500, in 41C2:34G7 over a range of 1:20 to 1:200, and in 34G7:41C2 over a range of 1:10 to 1:1000. In a four mcAb mixture, it is preferred that the three mcAbs that exhibit unique specificity and anti-viral activity directed at different HCMV glycoproteins be combined in equimolar ratios within a range of 100:1 to 1:100 for all components. In particular, it is preferred that the ratio of the embodiment MM-HCMV-9B7: MM-HCMV-F15-3B10: MM-HCMV-15F9 is 1:1:1. It is further preferred that the pair of augmenting gCI-specific mcAbs, in particular MM-HCMV-9B7:MM-HCMV-41C2, are combined in a ratio within a range of 100:1 to 1:100 depending on the optimal ratio that will achieve maximum synergistic anti-viral activity of the two mcAbs alone and in combination with other mcAbs in the cocktail.

The invention also provides for use of the cocktail composition for <u>in vitro</u> detection of HCMV infection. The synergistic binding activity of a combination of immunoglobulins in the cocktail composition can be used to increase the sensitivity of detection of extracellular HCMV virions in clinical specimens (i.e., urine, saliva, bronchoalveolar lavage fluid) and HCMV transplant organs, blood products, or biopsy/autopsy specimens over any single immunoglobulin.

The combination of a primary immunoglobulin labeled with a suitable detection molecule together with one or more augmenting immunoglobulins which increase the binding of the primary antibody to its target antigen can be used in any immunoassay format, including radioimmunoassay (RIA), enzyme-linked immunosorbant assay (ELISA), or immunofluorescence (IF) to increase the sensitivity of detecting HCMV virions and glycoproteins.

The invention also provides for a pharmaceutical composition comprising an amount of the immunoglobulin mixture which is effective to inhibit HCMV infectivity in a patient, when administered thereto, in combination with a pharmaceutically-acceptable vehicle such as a liquid carrier. The pharmaceutical composition is useful for treating patients with established HCMV infection and for preventing HCMV infection through prophylactic administration of the composition to high-risk individuals.

The carrier of the pharmaceutical composition may be any material which is otherwise inert, medically-acceptable and compatible with the active ingredients of the composition, and which assists in administering the immunoglobulin mixture to the patient. The carrier may be comprised of a physiologically balanced salt solution, with or without additional protein such as albumin, in order to reach a composition similar to that of human plasma suitable for parenteral intravenous or intramuscular administration. The carrier may incorporate buffers, extenders, solubilizing agents, stabilizing agents, and the like.

The pharmaceutical composition can be formulated as a powder, granules, solution, dispersion, aerosol, powder, or drops. The solution may be sterile, isotonic, and suitable for parenteral administration by intravenous, intramuscular, intraperitoneal, or subcutaneous injection. In addition to an effective

amount of the composition, the solution may contain appropriate adjuvants, buffers, preservatives and salts. The solution is preferably aqueous. The powder or granular forms may be combined with a solution and with diluting, dispersing and/or surface active agents. Solutions such as nose drops may contain antoxidants, buffers and the like.

The invention further provides for methods of medical treatment of HCMV infection utilizing the pharmaceutical preparation of the immunoglobulin cocktail. One method of treatment involves administering an effective amount of the pharmaceutical preparation as a prophylactic therapy for patients who are highly susceptible to HCMV infection. In particular, the cocktail composition may be administered to organ and bone marrow transplant recipients, HIV-seropositive patients, pregnant women exposed to or infected with HCMV in order to prevent symptomatic congenital HCMV infection of the developing fetus, and other high-risk individuals. Another method of treatment entails therapeutic intravenous, intraperitoneal, subcutaneous or intramuscular administration of the pharmaceutical composition to patients with established HCMV infections including HCMV-seropositive patients and those with acquired immune deficiency (AIDS) and HCMV infections.

In vitro neutralization data indicate that the serum concentration of total murine immunoglobulin in patients should be maintained at greater than or equal to 1 ug/ml. Although it is ultimately the physician's decision as to the amount of the pharmaceutical composition to be administered to a patient, the composition may typically be administered in a single dose or in multiple doses at effective non-toxic dose levels of about 1 mg/kg/day of the cocktail per body weight, based on a half-life of at least eight hours and non-synergistic anti-viral activity by the cocktail. In

vitro comparisons show the activity of the cocktail combination of mcAbs at a level of more than 100 times that of HCMV hyperimmune globulin. This increased activity derives from combining only HCMV-specific mcAbs in the preparation and from including in the mixture immunoglobulins which synergistically immunoreact to augment the activity of a neutralizing mcAb. Where synergistic activity is demonstrated in vivo, either the quantity of mcAb cocktail administered per dose may be reduced or the interval between doses may be increased.

The pharmaceutical composition may be administered rectally, vaginally, ocularly, nasally, intravenously, intramuscularly, intraperitoneally, or subcutaneously.

A composition composed of a mixture of HCMV-specific monoclonal immunoglobulins has several advantages over the existing single HCMV-specific monoclonal antibody preparations or human HCMV hyperimmune globulin medications. Those treatments have met with limited success due to the nature of HCMV and its different phases of infection, that is its early extracellular phase and its late stage intracellular phase. Moreover, the occurrence of multiple strains and mutated forms of HCMV limits the therapeutic effect of administering preparations comprised of a single immunoglobulin.

One advantage of the cocktail composition is that activity against HCMV is extended from a single phase of HCMV infection to several phases. HCMV has a slow replication cycle compared to that of other herpes viruses and is highly cell-associated, making HCMV relatively inaccessible to humoral antibodies. Moreover, HCMV uses certain host cell enzymes for its replication rather than virus-specific enzymes which are involved in the activity of some antiviral drugs. The invention combines together multiple immunoglobulins which are directed at different phases of HCMV

infection, namely the extracellular phase which is associated with the early stages of infection and the intracellular phase which occurs in the late stages of actual infection of tissues. This feature of the anti-HCMV immunoglobulin cocktail makes the preparation useful for treating individuals with established HCMV infections by inhibiting spread of HCMV in the bloodstream and the progression of HCMV in infected organs, and also for preventing HCMV infections in high risk individuals such as transplant patients and those with AIDS.

This broad therapy against established HCMV infections, which is provided by incorporating both gCI-specific and gCII-specific immunoglobulins into the mixture, is a distinct improvement over existing treatments. Sera from patients recovering from HCMV infection have been shown to contain antibodies reactive with gp55, gp93 and gp130 of the gCI complex of HCMV, and with Group 1 and Group 2 glycoproteins in gCII complexes. In contrast, sera obtained from infants with symptomatic congenital HCMV infection and their mothers showed reactivity with gp130, gp55 and gp93 of the gCI glycoprotein complex but not with either Group 1 or Group 2 gCII glycoproteins. The presence of gCI-specific antibodies in the sera of both groups of patients suggests that antibodies specific to the gCI glycoprotein complex may not be sufficient for recovery from persistent HCMV infection. Furthermore, the lack of gCII antibodies in congenital HCMV infants with persistent viruria suggests an important role for these antibodies. Therefore, the present invention provides an improvement over existing preparations directed at established HCMV infections because it combines both gCI-specific and gCII-specific immunoglobulins into the cocktail composition.

The multi-immunoglobulin cocktail of the present invention also provides activity against

multiple strains of HCMV and decreases the incidence of resistant strains of HCMV which are likely to emerge during prolonged administration of a single HCMV immunoglobulin. It has been found that immunosuppressed patients are frequently infected with multiple rather than single strains of HCMV. Although most HCMV-specific mcAbs recognize cross-reactive antigenic determinants, HCMV envelope glycoprotein epitopes have been identified which are uniquely expressed by particular strains of HCMV or which have been deleted altogether in other strains. The mcAb cocktail provides activity not only against multiple strains of HCMV but virtually all naturally occurring strains of HCMV. As such the cocktail provides an improvement over existing preparations because it will be useful in treating all immunosuppressed patients, irrespective of how many strains of HCMV are present. In addition, in vitro propagation of HCMV in the presence of particular mcAbs has resulted in mcAb-resistant (mar)-mutant viruses which have selectively deleted the epitope recognized by that particular antibody. Assuming that such HCMV mutant viruses occur with a frequency of approximately 10^{-6}, the probability of mutant strains developing in patients receiving immunoprophylaxis with an mcAb cocktail embodiment wherein four separate epitopes are deleted is approximately 10^{-24}. Therefore, the cocktail composition of the invention is a distinct advantage over single mcAb formulations by virtue of the mixture which, because of its reactivity with unique epitopes of the HCMV envelope glycoprotein, virtually eliminates the possibility of HCMV viral resistance.

A further advantage of the composition of the invention is its increased level of activity against HCMV as compared to existing therapies. High-risk patients such as organ and bone marrow transplant recipients, patients with congenital or acquired immune deficiency diseases, and patients receiving

immunosuppressive drugs frequently develop life-threatening opportunistic HCMV infections which are unresponsive to currently available anti-viral drugs and HCMV-hyperimmune globulin. In vitro assays show a level of activity in the cocktail composition of 100 times that of HCMV hyperimmune globulin. As a result of this enhanced activity, the quantity of monoclonal antibody protein required to achieve a desired anti-viral effect is reduced, thereby decreasing the amount and/or frequency of antibody to be administered. This has the advantage of simplifying a dosage regime, minimizing the risks of allergic reactions, and reducing production costs.

The increased level of activity of the composition of the invention stems in part from its make-up. Unlike existing globulin preparations which, besides various other components, include antibodies directed at substances other than HCMV, the cocktail composition is comprised exclusively of HCMV-specific mcAbs. As such, the cocktail preparation is a distinct advantage over existing preparations because it is able to provide HCMV-specific antibodies in plasma and interstitial fluid at a level sufficient to inactivate HCMV as an exogenous virion and as an intracellular replicating virion.

The heightened activity of the cocktail is also a result of the synergism that occurs between certain members of the immunoglobulin mixture whereby the binding and neutralizing activity of at least one of the members is augmented. As a result of the enhanced activity, the total amount of immunoglobulin (Ig) protein which must be administered to attain a particular inhibiting effect against HCMV is reduced. This is particularly desirable in order to decrease the risk of human anti-mouse antibody (HAMA) response which occurs in the majority of patients receiving therapeutic murine antibodies and to a lesser extent in patients

receiving chimeric mouse-human monoclonal antibodies, human-human monoclonal antibodies, and $F(ab')_2$ fragments of murine and human monoclonal antibodies. It has been shown that HAMA responses reduce the half-life of murine mcAbs by altering the pharmokinetics and biodistribution of the therapeutic mcAb. It has been further shown that the incidence and extent of the HAMA response increases in patients receiving large amounts of murine Ig protein in multiple doses over an extended period of time. Since it is preferable, therefore, to limit the total amount of immunoglobulin protein which is administered, the composition of the present invention provides an advantage over existing preparations.

The anti-isotypic HAMA response to the mcAb cocktail combination is likely to be comparable to that of an equivalent amount of a single mcAb. In contrast, the human antibody response to four different idiotypic determinants on the hypervariable regions of F(ab) regions of the four mcAbs in the cocktail may be reduced as compared to the response to a larger quantity of a single HCMV-specific mcAb that expresses a single idiotope. Since anti-idiotypic antibodies are more likely to interfere with the biologically active sites on the therapeutic mcAb, a reduction in that HAMA response represents a significant advantage to the mcAb cocktail composition.

The cocktail of the present invention is especially effective in diagnosing and detecting the presence of HCMV in patients, transplant material, and blood products. By combining into one preparation, mcAbs that are active against different glycoproteins expressed by virtually all strains of HCMV, <u>in vitro</u> detection of HCMV virions is greatly enhanced over single HCMV-specific mcAb preparations. The increased sensitivity of the cocktail ensures that its use in a diagnostic test will provide detection of all strains of HCMV. Moreover, both extracellular virions in clinical

samples and HCMV glycoproteins expressed on the surface of infected tissues will be detected by the cocktail composition.

EXAMPLE 1

Methods for production and characterization of the HCMV-specific monoclonal antibodies in the cocktail.

Generation of monoclonal antibodies to gp55 of gCI

Monoclonal antibodies were generated by using purified Towne HCMV virions as the immunizing antigen. Adult BALB/c mice were immunized intraperitoneally with antigen emulsified in complete Freund's adjuvant, followed with three booster immunizations using saline-suspended immunogen given at three-week intervals. Three days following the final booster, the mouse was sacrificed and the spleen cells fused with SP2/0 - Ag14 myeloma cells (American Tissue Culture Collection) at a ratio of 4:1 using polyethylene glycol. Twenty-four hours later, the fused cells were distributed into 96 wells in Hepes-buffered Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 50 ug/ml garamycin, 13.6 ug/ml hypoxanthine, 0.4 UM aminopterin and 7.6 ug/ml thymidine. After two weeks in this medium, the cells were transferred to new medium supplemented as above but without aminopterin. Three to four weeks after cell fusion, supernatants from these cultures were assayed for antibodies specific to HCMV NP-40 extract by an ELISA assay. Cloning of cells from positive cultures was performed by limiting dilution using mouse thymocytes as feeder cells. Individual wells were screened microscopically for single colonies and assayed for antibody to HCMV NP-40 extract as above. Antibody-producing clones were expanded for production of ascites fluid in BALB/c mice.

Generation of monoclonal antibodies to gp93 of gCI

McAbs which recognize gp93 were made by using purified gp93 from Towne strain HCMV as the immunizing agent in BALB/c mice. The virus was grown on skin fibroblasts according to Kari, et al., J. Virology 60:345-352 (1986). Extracellular virus was partially purified from culture media by differential centrifugation. To purify gp93 for the purpose of making mcAbs, gCI complex was immunoaffinity purified from a non-ionic detergent extract of HCMV using a gp55-specific biotinylated mcAb and streptavidin-agarose according to Gretch et al., Anal. Biochem. 163:270-277 (1987). The gCI complex was reduced and separated by SDS-PAGE in a 9% polyacrylamide gel using the method of Laemmli, Nature 227:680-684 (1970). Proteins were detected by Commassie blue staining and the gp93 band was cut from the polyacrylamide gel. The glycoprotein was electroeluted from the gel slice and SDS was removed from the protein with a 0.5 ml column of Extracti-gel D (Pierce Chemical Co.). Mice were immunized with purified gp93 to make mcAbs. Production and characterization of mcAbs were done according to Kari et al. (1986). Two mcAbs were obtained: 3C2 (IgG_1) and 9F9 (IgG_1). MM-HCMV-F15-3B10 was derived by further subcloning of 3C2.

Generation of gCII monoclonal antibodies

Adult BALB/c mice were immunized with 100 ug of whole Towne strain HCMV emulsified in complete Freund adjuvant and given a booster immunization with 100 ug whole Towne HCMV at three weeks. After several weeks a final boost was given using 20 ug of gCII which was purified by anion exchange high performance liquid chromatography according to Kari et al., (1986). Three days following the final boost with gCII, the mice were sacrificed and the spleen cells were fused with Sp2-0-Ag14 myeloma cells. Cloning was done according to Kari

et al. (1986). To identify positive hybridomas, culture medium was assayed for antibody against gCII by enzyme linked immunosorbant assay. For this assay, purified gCII was fixed in 96 well dishes. Hybridomas were subcloned twice by limited dilution. All mcAbs used in these studies were subtyped and found to have a single subtype. Clonality was further assayed by agarose isoelectric focusing of purified mcAbs.

Purification of monoclonal antibodies

Monoclonal antibody from ascites was purified by high-performance hydroxylapatite chromatography, according to Juarez-Salinas et al., Biotechniques (May/June 1984).

EXAMPLE 2

Radioactive labeling of HCMV proteins and immunoprecipitations

Glycoproteins were radiolabeled with [^{14}C]GlcN or [^{3}H]Arg (New England Nuclear) according to Kari et al. (1986). Labeled proteins were extracted from either extracellular virus or from infected whole cells using a Tris buffer (50mM Tris, pH 7.5, 150 mM NaCl, 1mM phenylmethylsulfonyl fluoride (PMSF)) containing 1.0% NP-40. Insoluble material was removed from centrifugation. Monoclonal antibodies, biotinylated according to Gretch et al. (1987), were added to these extracts along with streptavidin agarose beads (BRL). These solutions were mixed for one hour at room temperature and centrifuged to pellet the agarose beads. After several washes with PBS containing 0.1% NP-40, bound proteins were solubilized with SDS-PAGE sample solubilization buffer by heating at 100° C for three minutes. After SDS-PAGE, radioactive proteins were detected by fluorography using ^{3}H Enhance (New England Nuclear).

SDS-PAGE and Western blot

SDS-PAGE was done with either 7% or 10% polyacrylamide gels following the method of Laemmli (1970). Proteins in these gels were electroblotted onto nitrocellulose paper and the paper was then blocked with 3% gelatin in Tris-phosphate buffered saline (TBS, 20 mM Tris, 500 mM NaCl, phenylmethylsulfonyl fluoride (PMSF) 2 mM, pH 7.5). Strips of the blocked paper were incubated overnight with mcAbs in ascites diluted 1 to 500 in TBS containing 1% gelatin. Strips were washed with TBS containing 0.05% Tween 20 and then with TBS before incubation with phosphatase labeled goat anti-mouse IgG or goat anti-human IgG (Kirkegaard and Perry, Gaithersburg, MD) diluted 1 to 1000 with TBS containing 1% gelatin. After one hour incubation the paper was washed and the substrate 5-bromo-4-chloro-3-indoyl phosphate in 0.1 M Tris buffer (Kirkegaard and Perry) was added. After visualization the reaction was stopped by immersing the strips in water.

Immunofluorescence

Reactivity of the mcAbs with wild type and laboratory adapted strains (Towne, AD169, Toledo) of HCMV and several unrelated viruses was determined in an indirect immunofluorescence assay. Adenovirus, HSV, VZV, and wild type strains of HCMV were obtained from clinical isolates. For immunofluorescence, virus-infected and uninfected skin fibroblast cultures on glass slides were fixed in cold acetone:methanol (v/v, 1:1). Fixed cultures were preincubated with normal porcine serum and washed with phosphate-buffered saline (PBS). All subsequent steps were done with PBS containing 0.1% NP-40. Cultures were incubated with HPLC-purified ascites mcAb or with tissue culture fluid for 30 minutes and washed with PBS NP-40 buffer. Cultures were then incubated with fluorescein isothiocyanate (FITC) labeled goat antimouse IgG

(Cappel, Malvern, Pennsylvania). Following a final wash, slides were examined with a Zeiss phase fluorescence microscope.

Cells infected with Towne strain HCMV were then examined as either living or fixed cells. For these experiments, cells in 6 well culture dishes were infected with Towne strain HCMV at a MOI of 3×10^{-4} or 3. With the low MOI, cultures were harvested at 12 days post infection, and at 4 and 7 days when an MOI of 3 was used. Living cultures were then stained by indirect immunofluorescence using HCMV-specific mcAbs to detect HCMV glycoproteins in the plasma membrane. Duplicate cultures which had been fixed with acetone: methanol were stained at the same time to detect internal HCMV glycoproteins. For staining, cells were incubated with HCMV-specific mcAbs in phosphate buffered saline (PBS) pH 7.5 at 37°C for 1 hour. Cultures were washed and incubated with fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin (Cooper Biomedical Inc., West Chester, Pa.). Following a final wash, cultures were examined with a Zeiss phase fluorescence microscope.

Neutralization assays

Monoclonal antibodies purified by hydroxylapatite HPLC were diluted in DMEM supplemented with 2% fetal bovine serum (FBS). Prior to infection, 600 plaque-forming units (pfu) of Towne and 300 pfu of Toledo strain HCMV were added to mcAb in a total volume of 0.4 ml of DMEM. Guinea pig complement (Pel-Freeze Biologicals, Rogers, Arkansas) was added to a final concentration of 2.0% in half the preparations; the remaining half received an equivalent volume of DMEM. Viral neutralization was allowed to proceed for 60 minutes at 37°C. The HCMV-mcAb mixture was added to confluent layers of skin fibroblasts in 6-well plates (Costar, Cambridge, Massachusetts) and incubated for another 60 minutes for the virus to adsorb. The virus

mixture was aspirated and 5 ml of DMEM supplemented with 2% FCS, garamycin, penicillin G, and 0.5% agarose (Sea Plaque, FMC Co., Rockland, Maryland) was added to each well. On day 8, the monolayers were fixed with 10% formalin in 70% ethanol, stained with methylene blue and the plaque counted. Viral neutralization was evaluated using the formula:

$$\% \text{ Plaque Reduction} = \frac{\text{Avg. max. pfu - test pfu x 100}}{\text{Avg. max. pfu}}$$

and is reported as the protein concentration required for a 50% reduction in plaque numbers.

Simultaneous two antibody binding assay

Antigens were coated onto Immunolon 96-well microtiter plates. For this assay the antigens used were purified Towne strain whole virion and HCMV Towne strain glycoprotein complexes extracted from infected cell membranes with 1.0% NP-40. Extracted glycoprotein complexes were used either intact or reduced and alkylated according to Kari et al. (1986). As a control, uninfected fibroblasts were extracted with NP-40 and proteins in these extracts were used to coat wells. To prevent interference from NP-40 on the ability of proteins to coat the wells, protein extracts were passed over a column of Extracti Gel-D (Pierce Chemical Co.) to remove NP-40. Wells were coated with the same amount of protein for each of the preparations used. Monoclonal antibodies used in this assay were purified by HPLC according to Kari et al. (1986) and biotinylated using biotin N-hydroxysuccinimide ester according to Gretch et al. (1987). A fixed amount of biotinylated antibody and varying amounts of a second unlabeled antibody were added simultaneously to each well and incubated for 90 minutes at room temperature. Wells were washed three times with PBST and peroxidase-

labeled streptavidin (Kirkegaard and Perry) diluted 1/2000 in PBST was added and incubated for 90 minutes. After washing with PBST, the substrate OPD was added and OD_{490} was read. The biotinylated antibody was titered beforehand to determine the amount of antibody needed to give an OD_{490} of 0.5 to 1.0 in the absence of a second unlabeled antibody. This assay was performed in duplicate and the data were reduced using the following formula:

percentage augmentation or inhibition

$=[(OD_{490}$ with 2nd mcAb)$-(OD_{490}$ without 2nd mcAb)$/(OD_{490}$ without 2nd mcAb)] x 100.

EXAMPLE 3

Monoclonal antibodies reactive with HCMV glycoproteins comprising the gCI and gCII complexes

Complement-dependent and -independent neutralizing HCMV-specific mcAbs that are reactive with gp130 and gp55 have been obtained, and mcAbs reactive with unique epitopes on gp130 and gp93 have been developed which exhibit complement-independent neutralizing activity. A simultaneous competitive ELISA assay has been used to identify eight gCI-specific mcAbs reactive with unique continuous or conformation-dependent epitopes in three domains comprising a major B-cell antigenic region of gp55 in gCI complexes, which is the subject of U.S. Patent Application Serial No. 07/083,502, filed on August 7, 1987, the disclosure of which is incorporated by reference herein. Individual mcAbs in Domains I and II either augment or inhibit the binding of other gp55-specific mcAbs, and exhibit similar effects on virus neutralization in a plaque reduction assay. Monoclonal antibodies that are reactive with conformation-dependent epitope(s) in

Domain III inhibit binding and neutralizing activity of all other gp55-specific mcAbs.

All of the gp55-specific mcAbs immunoprecipitate gCI complexes and react with gp130, gp55 and proteolytic cleavage fragments obtained from digestion with chymotrypsin in Western blot analysis but do not react with gp93. In contrast, gp93-specific mcAbs react with gp130 and gp93 but do not react with gp55. Both gp55-specific and gp93-specific mcAbs immunoprecipitate unreduced gCI complexes which indicates that the gCI complex contains both of those glycoproteins.

At least one gCII-specific mcAb has been described which variably exhibits complement-independent neutralizing activity against Towne strain HCMV and which has been shown to recognize an epitope uniquely expressed on gp47-52 of gCII complexes, as claimed in U.S. Patent Application Serial No. 06/933,789, filed on November 24, 1986, the disclosure of which is incorporated by reference herein. Two non-neutralizing mcAbs reactive with gp47-52 have been identified and designated as Group 1 gCII mcAbs, as claimed in U.S. Patent Application Serial No. 07/390,300, filed on August 7, 1989, the disclosure of which is incorporated by reference herein. A second group of gCII-specific mcAbs has been developed which reacts with gp38-47, gp90 and gp200 in the higher molecular weight gCII complexes, but which is non-reactive with gp47-52, as claimed in U.S. Patent Application Serial No. 07/390,300, filed on August 7, 1989, the disclosure of which is incorporated by reference herein. Of interest, Group 1 gCII mcAbs recognize membrane-associated glycoproteins, whereas Group 2 mcAbs recognize intracellular proteins. Antibodies that are directed against gCII glycoproteins are likely to inhibit virion adsorption and penetration, virion assembly, and/or cell-to-cell spread.

EXAMPLE 4

The human antibody response to HCMV glycoproteins comprising the gCI and gCII complexes

Sera from patients who recover from HCMV infection contain antibodies reactive with structural virion proteins and glycoproteins and which exhibit neutralizing activity in the presence or absence of complement in vitro. Convalescent sera were examined by immunoprecipitation or Western blot analysis using HCMV-infected cell lysates or whole virions as antigens. Among the proteins recognized were gp55 and gp130 of the gCI complex. Of interest, no human antibodies reactive with gp93 of gCI or any of the Group 1 or Group 2 gCII glycoproteins were detected using whole viral antigen preparations.

Purification of individual gCI and gCII glycoproteins prior to immunological analysis has confirmed the importance of those glycoproteins in the human antibody response in normal individuals. It has also lead to the detection of a deficient level of gCII-specific antibodies in infants with symptomatic congenital HCMV infection and in their mothers. Human HCMV-positive convalescent sera recognize B-cell epitope(s) on unreduced gCI complexes and proteolytic fragments, and also react with gp130, gp55, and gp93 in Western blot analysis. In addition, human sera immunoprecipitate unreduced gCII complexes, and most react with individual Group 1 and Group 2 gCII glycoproteins in Western blot analysis. However, one of eight HCMV-positive adult donors tested lacked antibodies reactive with Group 1 or Group 2 gCII glycoproteins.

Longitudinal serum specimens taken from infants, ranging in age from 1 to 63 months, with symptomatic congenital HCMV infection and persistent viruria, were examined for the presence of human antibodies to gCI and gCII glycoproteins. All infant sera contained antibodies reactive with gp130, gp55, and

gp93, but none contained antibodies reactive with Group 1 or Group 2 gCII glycoproteins. Infant sera obtained during the first six to nine months following birth were non-reactive with gCII glycoproteins, which indicates a possible deficit in the mother's antibody response to the gCII glycoproteins. In addition, sera taken 32 and 63 months postpartum from two mothers of infants with congenital HCMV infections did not contain gCII-specific antibodies but did have antibodies that were reactive with gCI glycoproteins. In contrast, gCII-specific antibodies were detected in sera taken from fraternal twins, one with asymptomatic congenital infection and the other with an HCMV infection acquired at six months of age. These data indicate that susceptibility to the pathogenic effects of HCMV may be associated with maternal/fetal non-responsiveness to gCII and that gCII-specific antibodies play an essential role in host defense. The presence of gCI-specific antibodies in all sera from congenitally-infected infants, and in particular, those with persistent viruria and permanent neurological impairment, indicates that these individual antibodies may not be sufficient for recovery from persistent infection due to HCMV.

EXAMPLE 5

Specific description of monoclonal antibodies comprising the monoclonal antibody cocktail

Rational for antibody selection

The gp55 (gCI)-specific mcAb MM-HCMV-9B7 was selected because it exhibits complement-dependent neutralizing activity *in vitro*, and can inactivate infectious virus during the extracellular phase of HCMV viremia associated with primary and reactivated HCMV infection. A second gp55 (gCI)-specific mcAb, MM-HCMV-41C2, was included because it recognizes an epitope on gp55 in close spatial proximity on the conformational structure to the epitope recognized by mcAb 9B7 and can augment the binding and neutralizing activity of the 9B7

neutralizing mcAb to increase the therapeutic effectiveness of that antibody. A third gCI-specific mcAb, MM-HCMV-F15-3B10, recognizes a complement-independent epitope on gp93, and can interfere with viral infectivity (i.e., block adsorption to the viral receptor expressed on target organs, prevent penetration of the virus into susceptible cells). Monoclonal antibody MM-HCMV-15F9 recognizes Group 2 gCII glycoproteins and may prevent virion assembly and/or cell-to-cell spread which makes this mcAb useful for treating established intracellular infection.

Characterization of the HCMV-specific monoclonal antibodies in the cocktail

Immunoglobulin subtype:

MM-HCMV-41C2 and MM-HCMV-F15-3B10 are IgG_1 mcAbs; MM-HCMV-9B7 is an IgG_{2b} mcAb; MM-HCMV-15F9 is an IgG_{2a} mcAb. Monoclonality was confirmed by isoelectric focusing.

Specificity:

All mcAbs were tested for reactivity with HCMV (glyco)proteins expressed in fibroblasts infected with several strains of HCMV, including laboratory adapted strains (Towne, AD169, Toledo), 8 wild strain isolates obtained from infants with congenital HCMV infection or adults with opportunistic HCMV infections, and several unrelated viruses including herpes simplex virus (HSV)1 and 2, varicella zoster virus (VZV), and adenoviruses types 2 and 5. All four mcAbs were reactive with the three laboratory adapted strains and eight wild strains of HCMV, but did not show cross-reactivity with proteins expressed in cells infected with HSV, VZV, or adenoviruses types 2 and 5, as determined by indirect immunofluorescence. In addition, all four mcAbs were tested for reactivity with a panel of cell lines of fibroblast, epithelial, and lymphoid origin, and with tissue sections from 10 different organs derived at

autopsy. No reactivity was observed which confirmed that the mcAbs were not cross-reactive with normal cellular proteins.

The gp55-specific mcAbs 97 and 41C2 immunoprecipitated unreduced gCI complexes, and also reacted with constituent glycoproteins gp130 and gp55 in Western blot analysis. The gp93-specific mcAbs 3B10 also immunoprecipitated unreduced gCI complexes, and reacted with constituent glycoproteins gp130 and gp93 in Western blot analysis. The gCII-specific mcAb 15F9 immunoprecipitated unreduced gCII complexes, and reacted with Group 2 gCII glycoproteins, gp38-47, gp90 and gp200 in Western blot analysis. 15F9 recognized perinuclear and cytoplasmic proteins in cells infected with Towne and AD169 strains of HCMV.

Neutralizing activity:

The neutralizing activity of the four mcAbs was examined in a quantitative plaque reduction assay using both Towne and AD169 strains of HCMV. gp55-specific mcAb 9B7 neutralized both strains in the presence of complement, whereas gp55-specific mcAb 41C2 was non-neutralizing. gp93-specific mcAb 3B10 neutralized Towne and AD169 strains of HCMV in the absence of complement. Monoclonal antibody 15F9 was non-neutralizing in the plaque reduction assay. Monoclonal antibody 9B7 exhibited greater than 50% plaque reduction at a concentration of approximately 1 ug/ml of antibody protein, a level similar to that of three human HCMV hyperimmune globulin preparations. Neutralizing activity was augmented 20-100 fold by the addition of 5 ug/ml of the non-neutralizing mcAb 41C2.

These mcAbs can be evaluated in microneutralization assays to determine reduction of plaque size as a reflection of inhibition of cell-to-cell spread, quantitative plaque inhibition as a reflection of virostatic activity, and complement-dependent lysis of chromium 51-labeled HCMV-infected

target cells alone (antibody mediated-cytolysis) or in the presence of peripheral blood mononuclear cells (antibody-dependent cellular cytotoxicity, (ADCC)) to further determine their anti-viral activity in vitro. The activities in each of these assays will indicate the appropriate functionalities mentioned above.

The specificity of these mcAbs for HCMV-infected tissues has been characterized by testing their reactivity with normal cell lines and tissues which are not infected with HCMV using an indirect immunofluorescence assay. None of these mcAbs reacted with normal cellular proteins expressed on uninfected cell lines of fibroblast, epithelial, or lymphoid origin; nor with tissues from 10 different organs obtained at autopsy.

Mechanisms of Anti-Viral Activity:

Assays may be used to determine the potential anti-viral activity of HCMV-specific mcAbs but the extent to which this data may be extrapolated in order to define their protective activity in a human host is not yet determined. In vitro neutralization assays may be performed by pre-incubating virus with antibody in the presence or absence of complement, followed by washing and inoculation of a cell culture system in the absence of the antibody to determine if there is any residual infectious virus. Indications are that complement-dependent neutralization involves physical destruction of the virion whereas complement-independent neutralization involves selective blocking of a protein essential to the infectivity of the virus. Complement-independent neutralization may include such activity as an antibody which blocks access of a virion envelope glycoprotein to a cellular receptor wherein adsorption is prevented, or an antibody which blocks a fusion protein wherein an infectious virus is stopped from penetrating host cells. Molecular analysis of the gene encoding the gCI glycoproteins indicates that the

glycoproteins performing those functions may be the analogue of a glycoprotein in herpes simplex virus which is involved in viral fusion.

Additional assays can be utilized to determine whether non-neutralizing mcAbs exhibit virostatic anti-viral activity; that is, whether a virus is non-infectious in the presence of the antibody but infectious once the antibody is removed. In maintaining a therapeutic level of anti-viral activity throughout a period of prophylaxis or treatment, such inhibitory antibodies may be as effective as those which are virocidal (i.e., lethal to the virus). Plaque size reduction assays indicate that gcII glycoproteins and complexes may play a critical role in virion assembly and/or cell-to-cell spread. Viral inhibition assays are thereby conducted to determine the capacity of the gcII-specific mcAbs (i.e., 15F9) to prevent cytopathogenicity in cell culture when continually present. In addition, all monoclonal antibodies are examined according to two cytotoxicity assays, one of which evaluates direct lysis of HCMV-infected cells in the presence or absence of complement, and the other which determines the capacity of the antibody to mediate lymphocyte killing of virus-infected cells. Such activities not only destroy the repository for replicating intracellular virus but also protect adjacent, uninfected cells.

EXAMPLE 6

Formulation of Pharmaceutical Cocktail

The formulation of the pharmaceutical cocktail is determined on the basis of in vitro reactivity of the mcAbs with multiple strains of HCMV. Various formulations of the cocktail can be made by combining the four mcAbs in different proportions. Different dilutions of these combinations can be evaluated in a quantitative plaque reduction assay using Towne strain HCMV and in microneutralization, viral inhibition, and

cytolytic assays to confirm anti-viral activity against multiple strains of HCMV.

Cells/tissues obtained from patients or from prospective transplant material or blood products can be examined in vitro for evidence of HCMV glycoproteins. Seropositive tissues and/or sera can then be examined for sensitivity to different concentrations of various mcAbs mixtures according to in vitro anti-viral assays.

The appropriate mixture of mcAbs, as determined by the in vitro neutralization assays, can then be combined with a sterile aqueous solution containing a concentration of salt to achieve the physiological concentration found in human plasma, or with a physiologically balanced salt solution containing protein (i.e., albumin) wherein the total concentration of protein including mcAb protein does not exceed the normal protein concentration in human plasma (i.e., 5 gm/100 ml.).

Production characteristics:

All hybridomas were subcloned at least two times by limiting dilution to ensure clonality, and were shown to stably produce HCMV-specific antibody at a level of greater than 5 mg/ml in mouse ascites. All biological and immunological characterization of these mcAbs were performed on purified IgG obtained from HPLC hydroxyl apatite chromatography.

EXAMPLE 7

"Humanized" HCMV-Specific Monoclonal Antibodies

The unique specificities and synergistic anti-viral activities of component murine HCMV-specific mcAbs in the cocktail can be engineered into "humanized" mcAbs according to established methods including human-mouse B cell heterohybridomas, human-human B cell hybridomas, B lymphoblastoid cell lines by EBV transformation, and transfectomas producing chimeric human-mouse immunoglobulin by genetic engineering.

The unique binding specificity and functional activity of the component mcAbs are dependent on the variable regions of the heavy and light chains of the immunoglobulin molecules. Consequently, comparable humanized mcAbs must either be generated by co-transfecting variable region genes derived from murine hybridomas producing mcAbs of identical specificity with human constant region immunoglobulin genes or by hybridization of human B cells producing antibodies of identical specificities by selective immunization and screening with the unique peptide. Since immunoglobulin molecules are bivalent, a chimeric immunoglobulin molecule can be generated exhibiting dual specificity (two sites each of which bind a different epitope). In addition, the anti-viral activity of certain mcAbs may reside in the variable region so that Fab or $F(ab')_2$ fragments may be used rather than whole immunoglobulin. Other mcAbs, however, may require functional determinants (i.e., complement fixation) on the constant region of the heavy chain (Fc fragment) in order to exhibit anti-viral activity. These properties should be retained in any humanized mcAb which is generated.

The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

International Application No: PCT/ - /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page ____, line ____ of the description [4]

A. IDENTIFICATION OF DEPOSIT [5]

Further deposits are identified on an additional sheet ☒ [6]

2-29-9B7 (hybridoma)

Name of depositary institution [4]

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Address of depositary institution (including postal code and country) [4]

611(P) Hammonds Ferry Road
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Date of deposit [5]	Accession Number [6]
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01 August 1988	IVI-10181

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WHAT IS CLAIMED IS:

1. A cocktail composition comprising: a mixture of at least two human cytomegalovirus-specific immunoglobulins each of which is capable of binding with a different segment of human cytomegalovirus envelope glycoproteins or complexes.

2. A cocktail composition according to claim 1 wherein the immunoglobulins are capable of reacting with different, non-competing epitopes on the same or different human cytomegalovirus envelope glycoproteins.

3. A cocktail composition according to claim 1 wherein one of the immunoglobulins is capable of causing enhancement of the binding capacity and anti-viral activity of at least one other member of the mixture.

4. A cocktail composition according to claim 1 wherein the immunoglobulins display activity against an early and late stage of human cytomegalovirus infection.

5. A cocktail composition according to claim 1 wherein the immunoglobulins are capable of exhibiting complement-dependent and complement-independent neutralizing reactivity.

6. A composition according to claim 1 wherein at least one immunoglobulin is reactive with gp55 of the gCI glycoprotein complex of human cytomegalovirus.

7. A composition according to claim 1 wherein two of the immunoglobulins react with gp55 of the gCI glycoprotein complex of human cytomegalovirus so

that the first immunoglobulin reaction augments the binding capacity and anti-viral activity of the second immunoglobulin.

8. A composition according to claim 1 wherein one immunoglobulin reacts with a non-neutralizing epitope on gp55 of the gCI glycoprotein complex of human cytomegalovirus located in close spatial proximity to a complement-dependent neutralizing epitope on gp55 of the gCI complex so that said reaction augments a reaction of the second immunoglobulin with the neutralizing epitope.

9. A composition according to claim 1 wherein at least one immunoglobulin is reactive with gp93 of the gCI glycoprotein complex of human cytomegalovirus.

10. A composition according to claim 1 wherein at least one immunoglobulin is reactive with a complement-independent neutralizing epitope on gp93 of the gCI glycoprotein complex of human cytomegalovirus.

11. A composition according to claim 1 wherein at least one immunoglobulin is reactive with Group 2 glycoproteins of the gCII glycoprotein complexes of human cytomegalovirus.

12. A composition according to claim 11 wherein the immunoglobulin is reactive with gp38-47, gp90, gp200, or a combination thereof.

13. A composition according to claim 1 wherein at least one immunoglobulin is reactive with gp55 of the gCI complex of human cytomegalovirus, at least one immunoglobulin is reactive with gp93 of the gCI complex of human cytomegalovirus, and at least one immunoglobulin is reactive with Group 2

glycoproteins of the gCII glycoprotein complexes of human cytomegalovirus.

14. A composition according to claim 1 wherein the immunoglobulins are selected from the group consisting of monoclonal antibodies, chimeric monoclonal antibodies, bi-specific rearranged antibodies, F(ab) or $F(ab')_2$ fragments alone or in combination with Fc fragments, human monoclonal antibodies having hypervariable segments equivalent to the hypervariable segments of a murine monoclonal antibody that is specifically immunoreactive with human cytomegalovirus, murine monoclonal antibodies, and any combination thereof.

15. A composition according to claim 1 wherein the mixture comprises at least one IgG_1 monoclonal antibody, at least one IgG_{2b} monoclonal antibody, and at least one IgG_{2a} monoclonal antibody.

16. A composition according to claim 15 wherein the monoclonal antibodies are MM-HCMV-9B7, MM-HCMV-41C2, MM-HCMV-F15-3B10, and MM-HCMV-15F9.

17. A composition according to claim 1 wherein the mixture is capable of neutralizing or inhibiting extracellular and intracellular human cytomegalovirus.

18. A composition according to claim 1 wherein the mixture is capable of neutralizing or inhibiting extracellular human cytomegalovirus in the early stage of human cytomegalovirus infection and of neutralizing or inhibiting intracellular human cytomegalovirus in the late stage of human cytomegalovirus infection.

19. A cocktail composition according to claim 1 for use as a pharmaceutical composition for treatment of human cytomegalovirus infections in humans comprising an effective amount of the mixture and a pharmaceutically-acceptable carrier.

20. A pharmaceutical composition according to claim 19 wherein the carrier is a liquid.

21. A method of therapeutic or prophylactic treatment of a patient having or subject to a human cytomegalovirus infection, comprising: administering to the patient a pharmaceutical composition of an effective amount of a mixture of at least two human cytomegalovirus-specific immunoglobulins in combination with a suitable carrier wherein each of the immunoglobulins is capable of binding with a different segment of human cytomegalovirus envelope glycoproteins or complexes.

22. A method according to claim 21 wherein the immunoglobulins are capable of reacting with different, non-competing epitopes on the same or different human cytomegalovirus envelope glycoproteins.

23. A method of medical treatment according to claim 21 wherein one of the immunoglobulins is capable of causing enhancement of the binding and anti-viral capacity of at least one other member of the mixture.

24. A method according to claim 21 wherein the immunoglobulins display activity against an early and late stage of human cytomegalovirus infection.

25. A method according to claim 21 wherein the immunoglobulins are capable of exhibiting complement-dependent and complement-independent neutralizing reactivity.

26. A method of medical treatment according to claim 21 wherein at least one immunoglobulin is reactive with gp55 of the gCI complex of human cytomegalovirus, at least one immunoglobulin is reactive with gp93 of the gCI complex of human cytomegalovirus, and at least one immunoglobulin is reactive with Group 2 glycoproteins of the gCII glycoprotein complexes of human cytomegalovirus.

27. A method of medical treatment according to claim 21 wherein the immunoglobulins are selected from the group consisting of monoclonal antibodies, chimeric monoclonal antibodies, bi-specific rearranged antibodies, F(ab) or $F(ab')_2$ fragments alone or in combination with Fc fragments, human monoclonal antibodies having hypervariable segments equivalent to the hypervariable segments of a murine monoclonal antibody that is specifically immunoreactive with human cytomegalovirus, murine monoclonal antibodies, and any combination thereof.

28. A method of medical treatment according to claim 21 wherein the immunoglobulins are MM-HCMV-9B7, MM-HCMV-41C2, MM-HCMV-F15-3B10, and MM-HCMV-15F9.

29. A method of medical treatment according to claim 1 wherein the composition is administered to a patient selected from the group consisting of HIV-seropositive patients, human cytomegalovirus-seronegative pregnant women exposed to or actively infected with human cytomegalovirus, patients at risk of opportunistic human cytomegalovirus

infections, organ or bone marrow transplant patients, and any combination thereof.

30. A method of assaying for the presence of human cytomegalovirus in a fluid or a tissue specimen obtained from a patient, comprising: combining the specimen with a composition of at least two HCMV-specific immunoglobulins in combination with a detection marker to form a bound complex carrying the marker wherein each of the immunoglobulins is capable of binding with a different segment of HCMV envelope glycoproteins or complexes, and detecting a signal from the detection marker carried by the bound complex, said signal indicating the reaction between the cytomegalovirus and at least one immunoglobulin.

31. A method according to claim 30 wherein the immunoglobulins are capable of reacting with different, non-competing epitopes on the same or different human cytomegalovirus envelope glycoproteins.

32. A method of detecting the presence of human cytomegalovirus according to claim 30 wherein one of the immunoglobulins is capable of causing enhancement of the binding capacity and antiviral activity of at least one other member of the mixture.

33. A method according to claim 30 wherein the immunoglobulins display activity against an early and late stage of human cytomegalovirus infection.

34. A method according to claim 30 wherein the immunoglobulins are capable of exhibiting

complement-dependent and complement-independent neutralizing reactivity.

35. A method of detecting the presence of human cytomegalovirus according to claim 30 wherein at least one immunoglobulin is reactive with gp55 of the gCI complex of human cytomegalovirus, at least one immunoglobulin is reactive with gp93 of the gCI complex of human cytomegalovirus, and at least one immunoglobulin is reactive with Group 2 glycoproteins of the gCII glycoprotein complexes of human cytomegalovirus.

36. A method of detecting the presence of human cytomegalovirus according to claim 30 wherein the immunoglobulins are selected from the group consisting of monoclonal antibodies, chimeric monoclonal antibodies, bi-specific rearranged antibodies, F(ab) or $F(ab')_2$ fragments alone or in combination with Fc fragments, human monoclonal antibodies having hypervariable segments equivalent to the hypervariable segments of a murine monoclonal antibody that is specifically immunoreactive with human cytomegalovirus, murine monoclonal antibodies, and any combination thereof.

37. A method of detecting the presence of human cytomegalovirus according to claim 30 wherein the immunoglobulins are MM-HCMV-9B7, MM-HCMV-41C2, MM-HCMV-F15-3B10, and MM-HCMV-15F9.

38. A method of detecting the presence of human cytomegalovirus according to claim 30 wherein at least one immunoglobulin is capable of causing enhancement of the binding and anti-viral capacity of at least one immunoglobulin labeled with a detection molecule.

39. A method of detecting the presence of human cytomegalovirus according to claim 30 wherein more than one strain of human cytomegalovirus is detected.

40. A method of detecting the presence of human cytomegalovirus according to claim 30 wherein extracellular human cytomegalovirus, human cytomegalovirus glycoproteins expressed on the surface of tissue, or a combination of the two are detected.

41. A method according to claim 30 wherein the combining and detecting steps incorporate an immunoassay format selected from the group consisting of radio-immunoassay, enzyme-linked immunosorbant assay, or immunofluorescence assay.

INTERNATIONAL SEARCH REPORT

International Applicat. No. PCT/US90/06017

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12Q 1/04; C12Q 1/70; G01N 33/536;A61K 39/245
USCL: 435/7.1; 435/5; 424/85.8; 530/387

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/7.1; 435/5; 424/85.8; 530/387

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,514,505 (Canfield et al.) 30 April 1985, see Col. 1, line 43-48 and Col. 2, line 44.	30-41
Y/X	US, A, 4,783,399 (Oldstone et al.) 08 November 1988, Col. 11 lines 57-68, Col. 14. line 28.	30-41 / 30-41
X/Y	Virology, Volume 164, issued June 1988, Tussenhop et al. "Epitope Analysis of Human Cytomegalovirus Glycoprotein Complexes using Murine Monoclonal Antibodies", pages 362-372. see entire document.	30-35,37-41 / 1-20,36
Y	Klin Wachenschr, Volume 65, issued 15 October 1987, Schmeiser et al., "Cytomegalovirus (MV) Infections In Patients Receiving CMV-IgG-Hyperimmunoglobulin Prophylaxis After Bone-Marrow-Transplantation, pages 967-974 see entire document.	19.20.21.22 23,29

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
23 January 1991	28 FEB 1991

International Searching Authority	Signature of Authorized Officer [14]
ISA/US	Esther Kepplinger

Form PCT/ISA/210 (second sheet) (rev. 11 87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Virology, Volume 133, issued February 1984, Heinz, et al., "Antibody Induced Conformational Changes Result in Enhanced Avidity of Antibodies To Different Antigenic Sites On the Tick Borne Encephalitis Virus Glycoprotein, pages 25-34, see entire document.	23,3,32
Y	Transplantation Proceedings, Volume XIX, No. 6, Suppl. 7, issued December 1987, Emanuel et al., "The Diagnostic, Propthylactic, and Therapeutic Uses of Monoclonal Antibodies to Human Cytomegalovirus", pages 132-137, see entire document.	19,20,21,22 24,29,30,31 33,34,40,41
Y	Journal General Virology, Volume 68, issued 1987, Masuho et al., "Human Monoclonal Antibodies Neutralizing Human Cytomegalovirus", pages 1457-1461, see entire document.	1,5,6,14,18 19,20,21,25
Y	Journal of Virology, Volume 62, No. 3, issued March 1988, Gretch et al. "Identification and Characterization of Three Distinct Families of Glycoprotein Complexes in the Envelopes of Human Cytomegalovirus", pages 875-881, see entire document.	1,2,4,7,8,9 10,11,12,13 14,15,16,17 18
Y	Journal of Virology, Volume 60, No. 2, issued November 1986, Kari et al., "Characterization of Monoclonal Antibodies Reactive to Several Biochemically Distinct Human Cytomegalovirus Glycoprotein Complexes, pages 345-352, see entire document.	1-18

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Journal of Virological Methods, Volume 14, issued August 1986, Redmond et al., "The Selection and Characterization of human Monoclonal Antibodies to human Cytomegalovirus", pages 9-24, see entire document.	1,2,4,9,10, 14,15,17,18
Y	Hybridoma, Volume 6, No. 3, issued June 1987, FURLINI et al., "Monoclonal Antibodies Directed to Two Groups of Viral Proteins Neutralize Human Cytomegalovirus In Vitro", pages 321-326, see entire document.	1,2,4-18
X	The Journal of Infectious Diseases, Volume 159, No. 3, issued March 1989, Foung et al, "Human Monoclonal Antibodies to Human Cytomegalovirus", pages 436-443, see page 436, columnland page 442, column 1.	1-3,11,12, 13,15,19-21, 29

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter [2] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [4]

This International Searching Authority found multiple inventions in this international application as follows:

I. Monoclonal antibody composition and method of use (in vivo treatment) (claims 1-29).

II. 2nd Method of use (assay) (claims 30-41).

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. Telephone Practice

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4 ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.